

Repellency of Callicarpenal and Intermedeol Against Workers of Imported Fire Ants (Hymenoptera: Formicidae)

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ABSTRACT Callicarpenal and intermedeol are two insect-repellent terpenoids isolated from leaves of American beautyberry (*Callicarpa americana* L.; Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.). The repellency of these two terpenoids against workers of red imported fire ants, *Solenopsis invicta* Buren, black imported fire ants, *Solenopsis richteri* Forel, and a hybrid of these two species was evaluated using digging bioassays. In a multiple choice digging bioassay using two colonies from each species and their hybrid, callicarpenal showed significant repellency at concentration as low as 50 ppm against both red imported fire ant colonies and 6.25 ppm against all black imported fire ant and hybrid colonies. Intermedeol showed significant repellency at concentration as low as 1.50 ppm against both red imported fire ant colonies and 6.25 ppm against all black imported fire ant and hybrid colonies. In total, 15 colonies, five colonies from each species and the hybrid, were tested on callicarpenal and intermedeol at 50 ppm in a two-choice digging bioassay. Both callicarpenal and intermedeol showed repellency against all colonies, and intermedeol showed significantly greater repellency than callicarpenal against both species and their hybrid.

KEY WORDS repellent, imported fire ants, *Solenopsis invicta* Buren, *Solenopsis richteri*, hybrid

Imported fire ants, including the red imported fire ant, *Solenopsis invicta* Buren, the black imported fire ant, *Solenopsis richteri* Forel, and their hybrid, are serious medical and agricultural pests in the United States (Lofgren et al. 1975; Adams et al. 1983, 1988; Lofgren 1986; Drees and Gold 2003). Chemical treatment has been the major component of red imported fire ant management (Drees and Gold 2003). With the concern of potential environmental contamination of insecticides, there has been increasing interest in research on nontoxic or less-toxic treatments, such as fire ant repellents. Fire ant repellents may be useful to prevent ants from invading sensitive areas, such as nursing homes and hospitals. In the United States, the federal government has enforced a quarantine to prevent imported fire ants from spreading into noninfested areas (<http://www.aphis.usda.gov/ppq/ispm/fireants/index.html>). Repellents can potentially be useful in quarantine treatment by preventing fire ants from reentering treated materials, including nursery stocks and soil-moving equipment. Fire ant workers tend to damage electrical equipment and destroy circuitry (Vinson and MacKay 1990), so repellents also

may be useful to exclude fire ants from such equipment.

Several materials and compounds have been reported as fire ant repellents (Blum et al. 1991; Kaakeh and Dutch 1992; Vander Meer et al. 1993, 1996, 1998; Oi and Williams 1996; Anderson et al. 2002; Chen 2005). Vander Meer et al. (1993) found octanoic acid excluded fire ants from pots of nursery plants, whereas Oi and Williams (1996) reported the repellency of bifenthrin and tefluthrin in potting soil against red imported fire ants. Anderson et al. (2002) found that sage (*Salvia* spp.) leaves, pine (*Pinus* spp.) needles, and a water suspension of cedar shavings were repellent to red imported fire ants. Also, Chen (2005) discovered that dimethyl and diethyl phthalates were repellents to red imported fire ants. Appel et al. (2004) tested repellency and toxicity of mint oil granules, and they found that all red imported fire ant mounds treated with mint oil granules were abandoned.

Callicarpenal and intermedeol are two terpenoids isolated from the leaves of American beautyberry (*Callicarpa americana* L.; Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.). Both compounds are very active mosquito deterrents (Cantrell et al. 2005) and tick repellents (Carroll et al. 2007). The repellency of these two compounds to imported fire ants has never been tested. In this study, the repellency of callicarpenal and intermedeol against workers of red imported fire ants, black imported fire ants, and their hybrid was evaluated using an ant digging bioassay.

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Materials and Methods

Ants. Two sets of bioassays were conducted in this study: multiple-choice and two-choice bioassays. For the multiple-choice bioassay that was conducted in 2005, two colonies from each species and their hybrid were used. Red imported fire ant colonies were collected on 9 September in Sharkey County, MS, and black imported fire ant colonies were collected on 15 August in Granada County, MS. Two colonies of the hybrid (*S. invicta* × *S. richteri*) were collected on 9 September in Sunflower County, MS. For the two-choice bioassay that was conducted in 2007, five colonies from each species and the hybrid were used. Red imported fire ant colonies were collected on 22 February in Sharkey County, MS, and black imported fire ant colonies were collected on 20 March in Granada County, MS. Hybrid colonies were collected on 27 March in Granada County, MS. Fire ant mounds were shoveled and placed in a 19-liter plastic bucket. The inside wall of the bucket was then coated with baby powder (Cumberland Swan Holdings, Inc., Smyrna, TN) to prevent ant escape. Following the water-drip method developed by Banks et al. (1981), ants were separated and then placed in a 44.5- by 60.0- by 13.0-cm plastic tray with inside walls coated with Fluon (Ag Fluoropolymers, Chadds Ford, PA). Distilled water and 15% (wt:vol) sucrose water solution in separated test tubes that were plugged with cotton balls were placed in the trays. *Helicoverpa zea* (Boddie) and tobacco budworm, *Heliothis virescens* (F.), pupae, or adults of house cricket, *Acheta domestica* L., were used as additional food sources. One to three 14.0- by 2.0-cm petri dishes with 1.0 cm of hardened dental plaster (Castone; Dentsply International Inc., York, PA) on the bottom were placed inside each tray. In the petri dish was a 5.0-cm-diameter brood chamber. Two 8-mm access holes were made on the wall of the petri dish above the dental plaster. The petri dish lid was painted black (1302 Gloss Black Spray Enamel, Progress Paint Mfg. Co., Inc., Louisville, KY) to block light. All colonies were maintained at 22–25°C and a photoperiod of 12:12 (L:D) h.

Red and black imported fire ants are closely related species. Fortunately these two species and their hybrid can be readily separated using profiles of worker venom alkaloids and cuticular hydrocarbons (Vander Meer et al. 1985, Ross et al. 1987). The separation of species and the hybrid followed the method described by Ross et al. (1987). The social form of red imported fire ant colonies was determined using polymerase chain reaction (PCR). Primers described in Valles and Porter (2003) were used to amplify *Gp-9* alleles, indicating monogyne or polygyne colony status. Specimen collection, DNA extraction, and PCR methods were the same as described by Chen and Allen (2006). The social forms of black imported fire ants and the hybrid were not determined because no parallel molecular biological method was available to determine the social forms for *S. richteri* and hybrid as that for red imported fire ants.

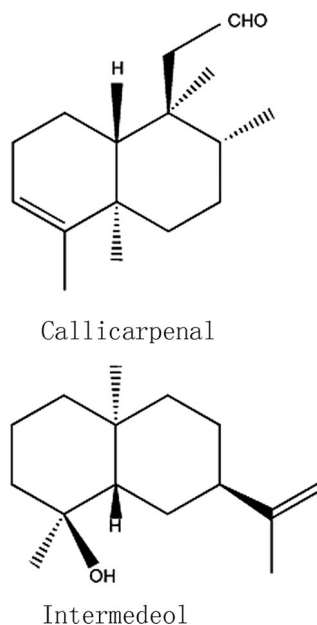


Fig. 1. Chemical structures of callicarpenal and intermedeol.

Chemicals. Callicarpenal and intermedeol (Fig. 1) were isolated from American beautyberry leaves following the method described by Cantrell et al. (2005). Purity of both callicarpenal and intermedeol samples was determined to be >95% by both gas chromatography-flame ionization detection analysis and ¹H and ¹³C NMR analysis. Dichloromethane (99.9% purity, American Chemical Society HPLC grade), used as a solvent for all test compounds, were purchased from Sigma-Aldrich (St. Louis, MO).

Multiple-Choice Bioassay. Chen (2005) developed a bioassay using fire ant digging behavior to evaluate chemical repellency. In his bioassay, the treatments were vials filled with moistened sand that was treated with active ingredient and the control was a vial with the sand that was only treated with solvent. Each vial had an entry hole on the cap. Treatments and control were presented to ants in a digging arena, such as in an aluminum pan. Fire ant workers dug and removed sand from the vials through the entry hole. The differences in amount of sand removed from the treated and control vials were used to evaluate chemical repellency. If ants dug more in treated sand, they would dig less in the control and vice versa, so if the tested compound was a repellent, it was expected ants would dig significantly more sand from the control. Repellants was defined as something that causes insects to make oriented movements away from its sources (Weldon 2004); however, as White (2007) pointed out; the term repellent has lost much of its original technical meaning due to its generalized use to refer to a formulated product or a chemical with a specific behavioral effect. Sometime, more precise terms are needed to describe the effects of chemicals on specific behaviors, or a more specific definition for repellent is

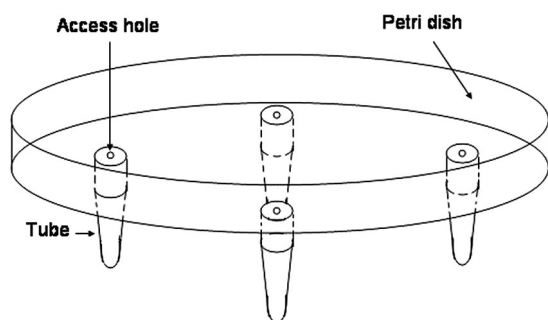


Fig. 2. Bioassay apparatus for evaluating fire ant repellents.

needed. Repellency in this study was defined as suppression of ant digging behavior. In a broad sense, repellency can be exerted by both olfactory and contact stimulants. Choice tests were usually used in fire ant repellency bioassays, such as the Y-tube olfactometer method that was designed for measuring olfactory stimuli (Vander Meer et al. 1996, 1998). The digging bioassay used in this study is also a choice test that measures olfactory stimulus, contact stimulus or both; however, this bioassay is apparently not able to differentiate these three possibilities. The setup of Chen's digging bioassay was modified in this study. The bioassay apparatus is shown in Fig. 2. Four 2-ml centrifuge tubes were mounted under a 14.0- by 2.3-cm petri dish by using glue (Arrow Fastener Co., Inc., Saddle Brook, NJ). Tubes were positioned 5.0 cm away from the center of the petri dish and at equal distance from each other. A 3-mm-diameter access hole was drilled for each centrifuge tube, which went through the bottom of the petri dish and the cap of the tube. The inner side of the petri dish was coated with Fluon. Three concentrations of a test compound and one control were set up in each apparatus. The experiment was replicated five times for each colony. The experiment was a randomized complete block design with three concentrations and a control and block on apparatus. Six concentrations, including 0.75, 1.50, 3.15, 6.25, 12.50, and 25.00 ppm were tested in two separated bioassays for each species and hybrid. Concentrations were expressed on dry-sand basis and calculated using the weights of test chemical and dry sand. One bioassay tested 0.75, 1.50, and 3.15 ppm and the other tested 6.25, 12.50, and 25.00 ppm. Because callicarpenal did not show the repellency against red imported fire ants, three higher concentrations of callicarpenal, 50.00, 100.00, and 150.00 ppm, were tested on red imported fire ants. Sand (Premium Play Sand, Plassein International, Longview, TX) was first sieved through a #35 U.S.A. standard testing sieve (Thomas Scientific, Swedesboro, NJ) and then washed with distilled water and dried at 350°C for 12 h. A 3-ml dichloromethane solution of callicarpenal or intermedeol was mixed with 30 g of sand in an aluminum pan. The sand was stirred every 2 min to facilitate the evaporation of the solvent under a fume hood. After dichloromethane evaporated (5 min), 1.92 ml

of distilled water was added and mixed with sand. Sand in the control tube was treated only with dichloromethane. In each tube, 2.78 ± 0.06 g (mean \pm SD) of wet sand was added. There was no open space inside the tube. Locations of each concentration were randomized. Fifty fire ant workers were introduced into the center of the petri dish. The experiment was conducted at $22 \pm 0.8^\circ\text{C}$ (mean \pm SD) temperature and $45.4 \pm 11.87\%$ RH. After 24 h, sand in each vial was collected, dried at 150°C for at least 4 h, and weighed. Two colonies of each species and the hybrid were tested. Both red imported fire ant colonies were monogyne. The analysis of variance (ANOVA) followed by least significant difference (LSD) mean comparison at $\alpha = 0.05$ (PROC Mixed, SAS Institute 1999) were used to compare the amount of sand removed by ants among treatments.

Two-Choice Bioassay. In total, 15 additional colonies, five colonies from each species and the hybrid, were tested in a two choice digging bioassay on callicarpenal and intermedeol. Five red imported fire ant colonies included three polygyne and two monogyne. The experiment was a completely randomized design of a factorial treatment structure with three ant categories and two chemicals. The bioassay apparatus was similar to that in multiple-choice bioassay except only two choices were presented in each bioassay apparatus: one was sand treated with callicarpenal or intermedeol and the other with solvent as a control. Two centrifuge tubes with access holes were mounted under a smaller petri dish (8.5 by 2.3 cm), and they were positioned 3.0 cm away from the center of the petri dish and at equal distance from each other. The other two tubes without access holes were used to support the bioassay device. Preparation of sand was the same as described above except that 10 ml of dichloromethane solution of callicarpenal or intermedeol was mixed with 100 g sand in an aluminum pan and 6.4 ml of distilled water was used. Locations of control and treatment were randomized. Twenty-five fire ant workers were introduced into the center of the petri dish. Each colony was replicated five times. After 24 h, sand in each vial was collected, dried at 150°C for at least 4 h, and weighed. To compare the repellency between chemicals and among species, two chemicals were tested at 50 ppm that showed repellency against both species and the hybrid in multiple-choice bioassays. Digging suppress index was used to compare the repellency, calculated using formula $I = (A_c - A_t) / (A_c + A_t)$, where I is the digging suppress index, and A_c and A_t are the amounts of sand removed from control tube and treatment tube, respectively. For each colony, a paired t -test was used to compare mean amount of removed sand between treatment and control. The ANOVA followed by LSD mean comparison at $\alpha = 0.05$ (PROC Mixed, SAS Institute 1999) was used to compare digging suppress index between two chemicals and among species and the hybrid.

Table 1. Mean \pm SE weight (grams) of sand removed by ants 24 h after release in the multiple-choice digging bioassay on callicarpenal against fire ant workers

Ant category	Colony	Concn. (ppm)	Sand removed (SE)	F value	P value
<i>S. invicta</i>	1	Control	0.30 (0.13)	0.40	0.76
		3.15	0.40 (0.20)		
		1.50	0.24 (0.15)		
		0.75	0.19 (0.07)	1.64	0.22
		Control	0.22 (0.13)		
		25.00	0.08 (0.05)		
	2	12.50	0.48 (0.19)	107.64	<0.0001
		6.25	0.21 (0.12)		
		Control	0.84 (0.05)		
		150.00	0.03 (0.007)*	0.03	0.99
		100.00	0.02 (0.008)*		
		50.00	0.04 (0.004)*		
<i>S. invicta</i>	2	Control	0.36 (0.12)	1.16	0.36
		3.15	0.36 (0.11)		
		1.50	0.32 (0.15)		
		0.75	0.32 (0.16)	11.32	0.0003
		Control	0.33 (0.19)		
		25.00	0.02 (0.01)		
	1	12.50	0.45 (0.18)	0.82	0.50
		6.25	0.24 (0.21)		
		Control	0.60 (0.15)		
		150.00	0.07 (0.03)*	22.85	<0.0001
		100.00	0.08 (0.06)*		
		50.00	0.02 (0.007)*		
<i>S. richteri</i>	2	Control	0.50 (0.16)	3.28	0.05
		3.15	0.21 (0.13)		
		1.50	0.28 (0.12)		
		0.75	0.40 (0.16)	140.12	<0.0001
		Control	1.03 (0.05)		
		25.00	0.08 (0.04)*		
<i>S. richteri</i>	1	12.50	0.40 (0.17)*	3.82	0.031
		6.25	0.10 (0.04)*		
		Control	0.82 (0.03)		
		25.00	0.01 (0.002)*	16.06	0.0002
		12.50	0.10 (0.04)*		
		6.25	0.10 (0.04)*		
Hybrid	1	Control	0.68 (0.04)	2.57	0.103
		3.15	0.30 (0.11)*		
		1.50	0.59 (0.10)		
		0.75	0.66 (0.09)	13.09	0.0004
		Control	1.00 (0.03)		
		25.00	0.39 (0.14)*		
Hybrid	2	12.50	0.26 (0.05)*	2.57	0.103
		6.25	0.53 (0.13)*		
		Control	0.69 (0.09)		
		3.15	0.58 (0.12)	13.09	0.0004
		1.50	0.36 (0.09)		
		0.75	0.53 (0.14)		
Hybrid	2	Control	0.92 (0.06)	2.57	0.103
		25.00	0.21 (0.11)*		
		12.50	0.30 (0.11)*		
		6.25	0.47 (0.09)*	13.09	0.0004
		Control	0.92 (0.06)		
		25.00	0.21 (0.11)*		

* Significantly different from the control (0.00 ppm).

Results

Multiple-Choice Bioassay. For red imported fire ants, at the 0.75, 1.50, 3.15, 6.25, 12.50, and 25.00 ppm levels, no significant effect of callicarpenal on the amount of removed sand was detected for both colonies (Table 1). At 50.00, 100.00, and 150.00 ppm levels, significantly less sand was removed from each

Table 2. Mean \pm SE weight (grams) of sand removed by ants 24 h after release in the multiple-choice digging bioassay on intermedeol against fire ant workers

Ant category	Colony	Concn. (ppm)	Sand removed (SE)	F value	P value
<i>S. invicta</i>	1	Control	0.68 (0.04)	11.61	0.0007
		3.15	0.20 (0.06)*		
		1.50	0.36 (0.09)*		
		0.75	0.56 (0.06)	14.20	0.0003
		Control	1.13 (0.13)		
		25.00	0.18 (0.09)*		
<i>S. invicta</i>	2	12.50	0.25 (0.13)*	15.93	0.0002
		6.25	0.66 (0.15)*		
		Control	0.85 (0.02)		
		3.15	0.35 (0.12)*	25.47	<0.0001
		1.50	0.35 (0.08)*		
		0.75	0.44 (0.10)*		
<i>S. richteri</i>	1	Control	0.74 (0.11)	1.91	0.17
		25.00	0.04 (0.01)*		
		12.50	0.02 (0.01)*		
	2	6.25	0.17 (0.08)*	31.21	<0.0001
		Control	0.82 (0.08)		
		25.00	0.04 (0.01)*		
<i>S. richteri</i>	1	12.50	0.07 (0.04)*	1.83	0.18
		6.25	0.16 (0.10)*		
		Control	0.70 (0.27)		
	2	3.15	0.17 (0.16)	127.06	<0.0001
		1.50	0.26 (0.17)		
		0.75	0.16 (0.12)		
Hybrid	1	Control	0.96 (0.04)	11.58	0.0003
		25.00	0.02 (0.02)*		
		12.50	0.14 (0.07)*		
	2	6.25	0.02 (0.01)*	27.04	<0.0001
		Control	0.98 (0.08)		
		25.00	0.20 (0.09)*		
Hybrid	1	12.50	0.37 (0.04)*	1.61	0.23
		6.25	0.45 (0.08)*		
		Control	0.59 (0.15)		
	2	3.15	0.28 (0.09)	49.25	<0.0001
		1.50	0.25 (0.10)		
		0.75	0.34 (0.14)		
Hybrid	2	Control	0.85 (0.07)	49.25	<0.0001
		25.00	0.04 (0.01)*		
		12.50	0.10 (0.04)*		
	1	6.25	0.20 (0.08)*	49.25	<0.0001
		Control	0.85 (0.07)		
		25.00	0.04 (0.01)*		

* Significantly different from the control (0.00 ppm).

of three treatments than the control for both colonies (Table 1). Intermedeol significantly reduced the amount of removed sand at 1.5 and 3.15 ppm levels for colony 1 and at 0.75, 1.5, and 3.15 ppm levels for colony 2. It significantly reduced the amount of removed sand at 6.25, 12.50, and 25.00 ppm levels for both colonies (Table 2).

For black imported fire ants, at the 0.75, 1.50, and 3.15 ppm levels, no significant effect of callicarpenal on digging effort was detected for colony 1; however, significant effect was detected at 3.15 ppm for colony 2 (Table 1). At 6.25, 12.50, and 25 ppm, callicarpenal significantly reduced the amount of removed sand for both colonies (Table 1). At 0.75,

Table 3. Mean \pm SE weight (grams) of sand removed by ants 24 h after release in the two-choice digging bioassay on callicarpenal and intermedeol at 50 ppm against imported fire ant workers

Chemical	Ant category	Colony	Sand removed (SE)		Digging suppress index (SE)	<i>t</i> value	<i>P</i> value
			Treatment	Control			
Callicarpenal	<i>S. invicta</i>	1	0.25 (0.1)	0.62 (0.05)	0.52 (0.17)	3.92	0.02
		2	0.13 (0.04)	0.58 (0.07)	0.65 (0.12)	5.09	0.007
		3	0.30 (0.07)	0.58 (0.05)	0.35 (0.14)	3.20	0.03
		4	0.09 (0.06)	0.58 (0.06)	0.74 (0.18)	4.37	0.01
		5	0.08 (0.05)	0.64 (0.09)	0.83 (0.10)	7.80	0.002
	<i>S. richteri</i>	1	0.10 (0.02)	1.11 (0.10)	0.84 (0.02)	10.66	0.0004
		2	0.23 (0.10)	0.78 (0.06)	0.61 (0.16)	5.20	0.0066
		3	0.80 (0.05)	1.40 (0.07)	0.27 (0.05)	5.83	0.004
		4	0.14 (0.13)	1.43 (0.16)	0.87 (0.12)	7.14	0.002
		5	0.64 (0.16)	1.47 (0.19)	0.41 (0.09)	4.23	0.013
	Hybrid	1	0.28 (0.10)	1.29 (0.06)	0.67 (0.10)	10.16	0.0005
		2	0.18 (0.10)	0.80 (0.09)	0.65 (0.19)	3.53	0.024
		3	0.27 (0.15)	1.02 (0.14)	0.70 (0.16)	5.71	0.0046
		4	0.33 (0.14)	1.04 (0.12)	0.60 (0.15)	5.45	0.006
		5	0.12 (0.05)	1.14 (0.07)	0.82 (0.07)	14.16	0.0001
Intermedeol	<i>S. invicta</i>	1	0.03 (0.05)	0.46 (0.08)	0.91 (0.06)	6.06	0.037
		2	0.04 (0.04)	0.42 (0.07)	0.80 (0.14)	3.49	0.025
		3	0.01 (0.008)	0.46 (0.10)	0.91 (0.06)	4.67	0.01
		4	0.004 (0.004)	0.42 (0.04)	0.98 (0.02)	11.74	0.0003
		5	0.006 (0.005)	0.47 (0.08)	0.96 (0.04)	5.82	0.004
	<i>S. richteri</i>	1	0.15 (0.09)	1.38 (0.21)	0.85 (0.09)	6.79	0.0025
		2	0.08 (0.07)	0.71 (0.17)	0.84 (0.10)	4.35	0.01
		3	0.12 (0.08)	1.05 (0.09)	0.84 (0.10)	9.97	0.0006
		4	0.00 (0.00)	0.78 (0.07)	0.99 (0.01)	11.82	0.0003
		5	0.02 (0.02)	0.98 (0.06)	0.97 (0.03)	18.28	<0.0001
	Hybrid	1	0.25 (0.1)	0.62 (0.05)	0.85 (0.07)	3.92	0.02
		2	0.13 (0.04)	0.58 (0.07)	0.94 (0.03)	5.09	0.007
		3	0.30 (0.07)	0.58 (0.05)	0.83 (0.16)	3.20	0.03
		4	0.09 (0.06)	0.58 (0.06)	0.89 (0.10)	4.37	0.01
		5	0.08 (0.05)	0.64 (0.09)	0.96 (0.01)	7.80	0.002

1.50, and 3.15 ppm levels, no significant effect of intermedeol was detected for both colonies (Table 2). Intermedeol significantly reduced the amount of removed sand at 6.25, 12.50, and 25.00 ppm levels for both colonies (Table 2).

For the hybrid, at the 0.75, 1.50, and 3.15 ppm levels, no significant effect of callicarpenal on digging effort was detected for colony 2; however, significant effect was detected at 3.15 ppm for colony 1 (Table 1). Callicarpenal significantly reduced the amount of removed sand at 6.25, 12.50, and 25.00 ppm levels for both colonies (Table 1). At 1.50 and 3.15 ppm levels, significant effect of intermedeol on digging effort was detected for colony 1 but not for colony 2 (Table 2). At 6.25, 12.50, and 25 ppm, intermedeol significantly reduced the amount of removed sand for both colonies (Table 2).

Two-Choice Bioassay. At 50 ppm, for each colony, both chemicals significantly suppressed the digging behavior of imported fire ants (Table 3). There was a significant difference in digging suppress index between callicarpenal and intermedeol ($F = 31.42$; $df = 1, 12$; $P = 0.001$). Intermedeol was a stronger repellent than callicarpenal against both species and the hybrid. No significant difference was detected among ant categories for both compounds ($F = 0.18$; $df = 2, 12$; $P = 0.84$). Interaction between chemical and ant categories was also not significant ($F = 0.37$; $df = 2, 12$; $P = 0.70$).

Discussion

Callicarpenal is a newly identified terpenoid that is a bite deterrent against adult *Aedes aegypti* (L.) and *Anopheles stephensi* Liston mosquitoes (Cantrell et al. 2005). This study demonstrated that callicarpenal is also a repellent against the workers of red imported fire ant, black imported fire ants, and their hybrid.

Biological systems usually exhibit different responses to difference doses; however, in the multiple choice bioassay, ants sometimes did not show differential responses to the different doses. For example, in the test on callicarpenal against red imported fire ants, ants removed almost same amount of sand at concentrations of 1.50 and 0.75 ppm. This might just mean that ants were not able to differentiate the small difference between 0.75, and 1.50 ppm. If more widely separated doses were used, such as 0.75, 25, and 150 ppm in a bioassay, dose-response interaction by the ants might be easier to be detect.

Intermedeol has been found in essential oils from orange (Moshonas and Shaw 1979), Portuguese *Thymus albianus* (L.) (Miguel et al. 2004), *Cymbopogon afronardus* Stapf (Baser et al. 2005), *Ligularia fischeri* variety *spiciformis* (Ledeb.) Turcz. (Jeong et al. 2002), *Bothriochloa pertusa* (L.) A. Camus (Kaul and Vats 1998), and *Thymus camphorates* L. (Salgueiro et al. 1997). Intermedeol also was found in frontal gland secretion of termite soldiers, *Velocitermes velox* (Holmgren) (Valterova et al. 1988). The repellency of

intermedeol against fire ants has not been reported previously. The results of this study showed intermedeol to be a strong fire ant repellent, with significant repellency at concentrations as low as 1.50 ppm against red imported fire ants, 6.25 ppm against black imported fire ants, and 6.25 ppm against the hybrid. Comparison between two chemicals at 50 ppm by using the digging suppress index showed that intermedeol is a stronger repellent than callicarpal against both ant species and their hybrid. This differs from short-term dose-response tests with the black-legged tick, *Ixodes scapularis* Say, where nymphs showed no difference in repellency among callicarpal, intermedeol and deet (*N,N*-diethyl-3-methylbenzamide) (Carroll et al. 2007).

Vander Meer et al. (1993) have proposed applications of fire ant repellents in the following potential areas: 1) protection of biological control agents, 2) protection of electrical circuitry and switches, 3) exclusion of fire ants from hospitals, and 4) protection of trees by using repellent tree wraps. With evidence of strong repellency of intermedeol and callicarpal against fire ant workers in the laboratory, further investigation of the practical application of these two compounds in those areas is warranted.

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